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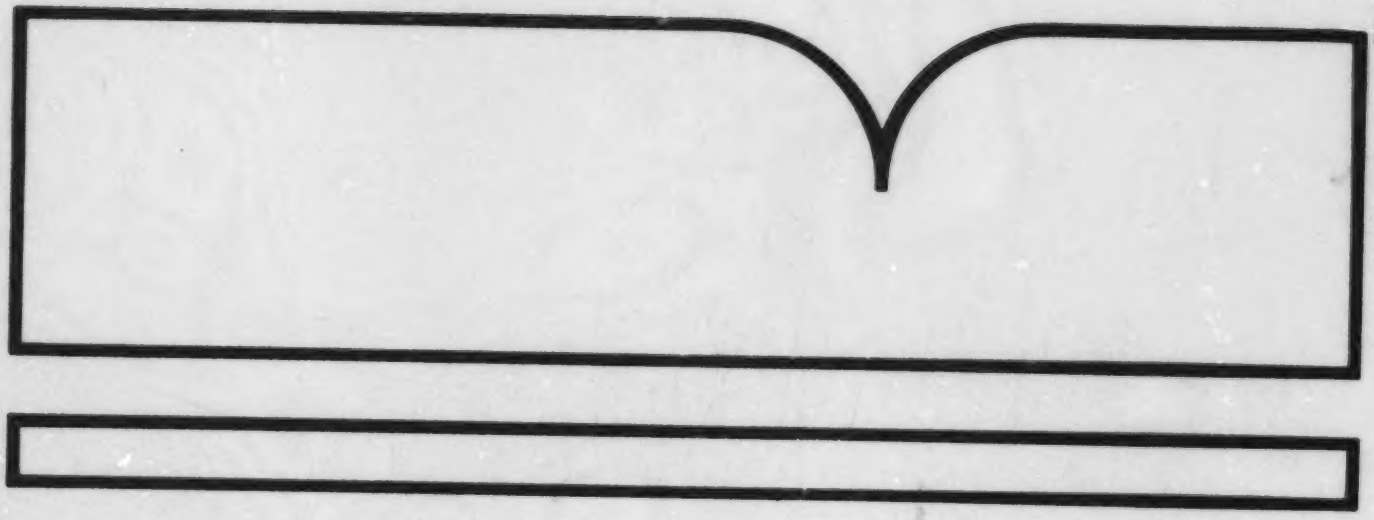

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FATE OF AZO DYES IN THE ACTIVATED SLUDGE PROCESS

by

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16. ABSTRACT

The U.S. Environmental Protection Agency's (EPA) Office of Toxic Substances (OTS) evaluates submissions to the Premanufacture Notification (PMN) process under section 5 of the Toxic Substances Control Act (TSCA). Azo dyes constitute a significant portion of these submissions. OTE is concerned because some of the dyes, dye precursors or their degradation products such as aromatic amines, which are also dye precursors, have been shown to be, or are suspected to be, carcinogenic. Therefore, EPA's Water Engineering Research Laboratory, Office of Research and Development has an ongoing study to determine the fate of specific azo dye compounds in the activated sludge process (ASP).

The objective of this study is to dose pilot plant ASP systems with various azo dyes and monitor each dye compound through the system, analyzing both liquid and sludge samples. The fate of the parent dye compound was assessed via mass balance calculations. From these data, it was possible to determine if the compound was removed by adsorption, apparent biodegradation or not removed at all. This report presents results to date (5/86) from this study which is being conducted at EPA's Test and Evaluation Facility in Cincinnati, Ohio.

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FATE OF AZO DYES IN THE ACTIVATED SLUDGE PROCESS

INTRODUCTION

The U. S. Environmental Protection Agency's (EPA) Office of Toxic Substances (OTS) evaluates submissions to the Premanufacture Notification (PMN) process under section 5 of the Toxic Sustances Control Act (TSCA). Azo dyes constitute a significant portion of these submissions. OTS is concerned because some of the dyes, dye precursors or their degradation products such as aromatic amines, which are also dye precursors, have been shown to be, or are suspected to be, carcinogenic [1]. Therefore, EPA's Water Engineering Research Laboratory, Office of Research and Development has an ongoing study to determine the fate of specific azo dye compounds in the activated sludge process (ASP).

The objective of this study is to dose pilot plant ASP systems with various azo dyes and monitor each dye compound through the system, analyzing both liquid and sludge samples. The fate of the parent dye compound was assessed via mass balance calculations. From these data, it was possible to determine if the compound was removed by adsorption, apparent biodegradation or not removed at all. This report presents results to date (5/86) from this study which is being conducted at EPA's Test and Evaluation Facility in Cincinnati, Ohio.

EXPERIMENTAL PROGRAM

A previous paper [2] described in detail the background, objectives and analytical methods employed for this study. This paper describes work that used wastewater from the Greater Cincinnati Mill Creek Sewage Treatment Plant as the influent to three pilot-scale ASP systems (two experimental and one operational control). Each dye was dosed as

commercial product to the two experimental systems operated in parallel at 1 and 5 mg/L of influent flow (henceforth referred to as the low and high spike systems, respectively). The principal focus of this work is on the ASP and as such the primary sludge was not sampled. These selected dosages were based on expected concentrations in publicly owned treatment works [2]. Table 1 presents a summary of the operating conditions of the pilot plant systems. Each system consisted of a primary clarifier, complete-mix aeration basin and secondary clarifier.

TABLE 1. SUMMARY OF OPERATING CONDITIONS

Parameter	Value
Influent Flow Rate, L/d	720
Primary Sludge Flow Rate, L/d	6
Primary Effluent Flow Rate, L/d	714
Mixed-Liquor Wastage Flow Rate, L/d	67
Secondary Effluent Flow Rate, L/d	647
Solids Retention Time, days	2.7
Hydraulic Retention Time, days	0.28
Dissolved Oxygen, mg/L	2.0-4.0
Influent Spike Dosages, mg/L	
Low	1
High	5
Influent pH, pH units	7.0-8.0
Aeration Basin Temperature, °C	21-25

Before each intensive data collection phase, dye recovery studies were conducted in organic free water, influent wastewater and mixed liquor. These studies were run in duplicate and each recovery study was repeated at least once in order to ensure that the compound could be extracted from these samples. Purified dye standards were analytically prepared from the commercial dye product as per Tincher [3] since standards are not available from conventional sources. All samples were analyzed by high performance liquid chromatography (HPLC) with an ultraviolet-visible detector. The targeted range of recovery from all samples was 80 to 120 percent. If recovery analyses were within this range, then it was assumed that little, if any, chemical transformation was occurring. Previous work had found

that recovery of the dye from sludge was less than the goal of 80 to 120 percent. However, through a change in the solvents used in the published methods [3,4] acceptable recoveries were achieved for sludge samples, and as such, all data reported herein are as measured and have not been corrected for recoveries. All samples were analyzed by the University of Cincinnati's Kettering Laboratory of the College of Medicine.

All systems were operated for at least three times the solids retention time (SRT) to ensure acclimation prior to intiation of data collection. During the intensive data collection period the following analytical tests were performed:

* Total suspended solids (SS) analyses on the influent (INF), primary effluent (PE), mixed liquor (ML) and activated sludge effluent (ASE) samples. All samples were analyzed using EPA approved methods [5].
* Specific dye analyses on the PE, ML and ASE samples. The ML samples were filtered through 0.45 micron pore size filters and the soluble dye in the filtrate (SOL) and suspended residue (RES) were analyzed as separate samples. On occasion, the INF samples were analyzed to ensure that targeted dosages were maintained. No standard method exists for these analyses, however Tincher [3] and Dickson [4] have developed an analytical method.

All samples were 24-hour composites made up of six grab samples collected every four hours and stored at 4 ^{o}C. Each test run was sufficiently long to allow at least six sets of samples collected over three to five weeks (after acclimation) for the specific dye analyses. If acclimation appeared to be continuing during the data collection phase, the intensive data collection period was usually extended accordingly.

In addition to the pilot plant studies, adsorption isotherms were developed for each of the dyes using activated sludge solids as the adsorbent. Purified dyes prepared from the commercial product were used in these studies in order to minimize interferences from the impurities of the commercial formulations. These studies were conducted with sludge solids obtained from the control pilot plant system which did not receive a dye spike. In order to prevent potential biodegradation from influencing the adsorption measurements, the biological solids were inactivated by freeze-drying of the solids with subsequent desiccation of the dry biomass in an oven for a minimum of three hours at 105° C. This procedure was reported [6] to be the simplest method for inactivation of the biomass while generally retaining the structural integrity of the biomass. Flocculation and settling properties of this freeze-dried, desiccated biomass resembled viable activated sludge solids upon rehydration. In addition, photomicrographs of this material revealed the presence of structurally-intact organisms.

Freeze-dried, desiccated biomass was utilized in kinetic studies to determine the contact time required for the test dyes and sludge solids to reach equilibrium. Previous studies have reported contact times of 40 to 120 minutes [7], 30 minutes [8] and 20 minutes [9] for some azo dyes. However, 24 hours was chosen as the standard contact time for all dyes investigated in this study since the kinetic experiments revealed that although for some dyes equilibrium was reached within one or two hours, other dyes require six or more hours of contact between the test dye and biomass for adsorption equilibrium to be attained.

Adsorption isotherm experiments were performed once the equilibrium contact time was established. A typical experimental set-up consisted of a

series of stoppered glass vessels which contained increasingly greater concentrations (typical range was 0.5 to 8.0 g/L solids concentrations) of the inactivated sludge solids which were rehydrated in distilled water. A known concentration of dye was spiked into each vessel and the contents of each were well mixed for 24 hours via magnetic stirring apparatus. A quality control sample included in this set-up consisted of a dye blank (no sludge solids) to assess potential changes in soluble dye concentration due to dye molecules adhering to the inside walls of the container or photodegradation. Another quality control sample was obtained from a vessel containing sludge solids in water which did not receive the dye spike. This was then used to assess potential analytical interferences orginating from the biomass. The contents of all vessels were sampled at the end of the test. These samples were centrifuged at 10,000 revolutions per minute for fifteen minutes and the centrate was subsequently filtered through a 0.45 micron pore size filter.

High performance liquid chromatography (HPLC) was used to analyze for the residual dye in the sample filtrates. The amount of residual dye was then utilized with the amount of biomass in each vessel to calculate an adsorption isotherm according to the Freundlich relationship. This is an empirically derived [10] relationship with the general form:

$$X/M = K C_e^{1/n} \qquad (1)$$

where:

X = amount of solute (dye) adsorbed, mg/L

M = concentration of adsorbent (sludge solids), g/L

X/M = amount of dye adsorbed per unit mass of sludge solids, mg/g

C_e = equilibrium concentration of dye in solution, mg/L

K,n = empirical constants

The logarithmic form of the Freundlich equation is employed to obtain a straight line relationship:

$$\log (X/M) = \log K + (1/n)(\log C_e) \qquad (2)$$

This linear form of the Freundlich model may be illustrated graphically with a plot of X/M versus C_e on log-log paper. The line of best fit was calculated using least squares regression analysis. The slope of this line (1/n) indicates adsorption intensity whereas the constant K indicates adsorption capacity.

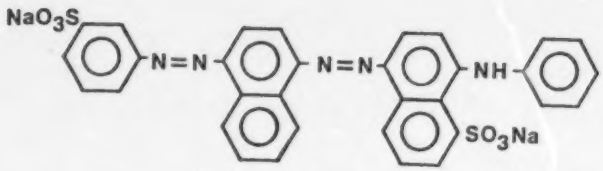
Other adsorption models could have been evaluated. For example, Langmuir's equation and the Brunauer-Emmett-Teller (BET) equation are commonly utilized in adsorption studies [11]. However, Dohanyos, et al [7] applied all three models to data for 22 textile dyes and found the Freundlich relationship to be the most suitable for 18 of these dyes with activated sludge solids as the adsorbent.

RESULTS AND DISCUSSION

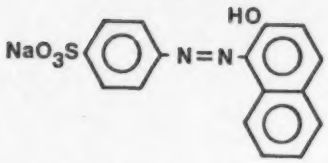
Seven acid azo dyes have been studied and are listed below by the Colour Index Name and Number [12] with Figure 1 presenting the chemical structure for each:

NAME	NUMBER	NAME	NUMBER
C.I. Acid Blue 113	26360	C.I. Acid Red 151	26900
C.I. Acid Orange 7	15510	C.I. Acid Red 337	-----
C.I. Acid Red 1	18050	C.I. Acid Yellow 151	13906
C.I. Acid Red 88	15620		

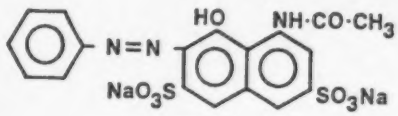
The possible removal mechanisms for a dye compound in the ASP system



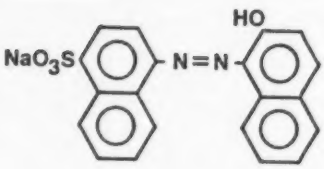
C.I. ACID BLUE 113



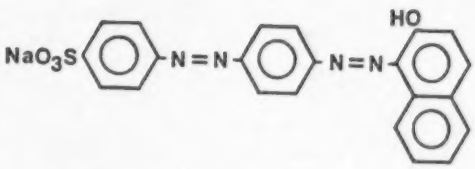
C.I. ACID ORANGE 7



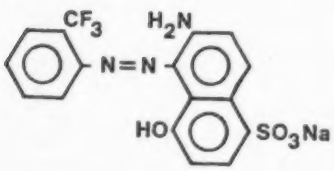
C.I. ACID RED 1



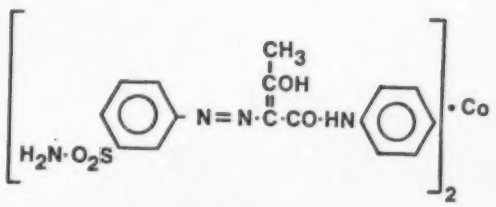
C.I. ACID RED 88



C.I. ACID RED 151



C.I. ACID RED 337



C.I. ACID YELLOW 151

Figure 1. Dye Structures

include adsorption, biodegradation, chemical transformation, photodegradation, and air stripping. During the spike recovery studies for each dye, several wastewater and sludge samples from the control ASP were spiked and recovery was assessed. Recovery for all dyes was within the targeted range of 80 to 120 percent; thus, it appeared that little or no chemical transformation occurred for any dye due to contact with the variable wastewater and/or sludge matrix. In addition, photodegradation potential was investigated with each dye added to distilled/deionized water and no measurable decrease in concentration was observed for samples held in light for more than two weeks. Moreover, the estimated Henry's law constant for each dye tested is less than 10^{-15} atm-m^3/mol and as such, air stripping is very unlikely [13]. Therefore, adsorption and/or biodegradation appeared to be the possible removal mechanisms.

In order to determine the fate of the parent dye compound in the ASP systems and to allow a comparison between the adsorption isotherm data and the ASP data, mass balance calculations were made. The mass balance calculations are presented below:

DYE IN = (PE FLOW RATE, L/d) (PE DYE CONC., mg/L) = mg/d

DYE OUT = (DYE OUT 1) + (DYE OUT 2)

DYE OUT 1 = (DYE OUT VIA SECONDARY EFFLUENT)

DYE OUT 2 = (DYE OUT VIA WASTING MIXED LIQUOR, WAS)

DYE OUT 1 = (ASE FLOW RATE, L/d) (ASE DYE CONC., mg/L) = mg/d

DYE OUT 2 = (WAS FLOW RATE, L/d) (WAS SOL DYE CONC., mg/L)
+ (WAS FLOW RATE, L/d) (WAS ADSORBED DYE CONC., mg/L) = mg/d

with

(WAS ADSORBED DYE CONC.) = (WAS SS CONC., g/L)(RES DYE CONC., mg/g)

DYE Recovered, % = (DYE OUT/DYE IN) x 100

Table 2 presents the mean SS and dye concentrations and standard deviations calculated from the ASP raw data, while Table 3 presents the mass balance closure data calculated from the values in Table 2. Upon inspection of the data from Table 2, it can be seen that wide fluctuations in dye removal (PE versus ASE) were measured. (Note that two runs were conducted for C.I. Acid Orange 7).

From Table 3, it can be seen that three of the seven dyes studied had total mass balance closures within the 80 to 120 percent range. This indicates that there was little or no biodegradation of these three dyes. The three dyes were C.I. Acid Red 1, C.I. Acid Red 337 and C.I. Acid Yellow 151. C.I. Acid Red 1 had very little adsorption onto the sludge with the majority of the dye compound exiting the ASP in the secondary effluent stream. Both C.I. Acid Red 337 and C.I. Acid Yellow 151 were found to be partially adsorbed (9 to 17 percent) onto the sludge; however, the majority of these dyes was found in the secondary effluent.

The data in Tables 2 and 3 show that only 4 to 27 percent and <1 to 7 percent of the influent dye could be accounted for in the ASE and WAS for C.I. Acid Orange 7 and C.I. Acid Red 88, respectively. Thus, it is apparent that both of these dyes underwent significant biological degradation.

Both C.I. Acid Blue 113 and C.I. Acid Red 151 were strongly adsorbed (see WAS RES Dye in Table 2). Between 22 and 33 percent of the amount of these dyes entering the aeration basin could not be accounted for in the ASE and WAS. Thus, there may have been some limited degradation of C.I. Acid Blue 113 and C.I. Acid Red 151.

Table 4 presents a summary of the adsorption isotherm data showing the

TABLE 2. Data Summary*

		Primary Effluent (PE)		Waste Activated Sludge (WAS)			Activated Sludge Effluent (ASE)		Percent Removal†
		SS mg/L	Total Dye mg/L	SS mg/L	SOL Dye mg/L	RES Dye mg/g	SS mg/L	Total Dye mg/L	%
C.I. Acid Blue 113									
Low Spike	Mean	190	0.84	1490	0.04	4.10	7	0.07	92
	Std. Dev.	61	0.20	330	0.02	2.43	3	0.03	
	Number	5	5	5	5	4	5	4	
High Spike	Mean	220	4.55	1150	0.44	20.65	13	0.84	82
	Std. Dev.	105	0.33	252	0.10	7.74	2	0.17	
	Number	5	4	5	5	4	5	5	
C.I. Acid Orange 7 Run 1									
Low Spike	Mean	140	0.95	950	0.15	<0.02	17	0.18	81
	Std. Dev.	20	0.06	240	0.20	-	20	0.19	
	Number	6	6	6	6	6	6	6	
High Spike	Mean	90	5.34	910	0.14	<0.02	21	0.24	96
	Std. Dev.	41	0.32	74	0.15	-	39	0.17	
	Number	6	6	6	6	6	6	6	
C.I. Acid Orange 7 Run 2									
Low Spike	Mean	160	1.07	1010	0.22	<0.03	21	0.29	73
	Std. Dev.	53	0.23	236	0.19	-	30	0.21	
	Number	10	10	10	10	10	10	10	
High Spike	Mean	220	5.57	970	0.53	<0.02	41	0.73	87
	Std. Dev.	69	1.03	230	0.61	-	43	0.67	
	Number	10	10	10	10	10	10	10	

TABLE 2. (Continued)

		Primary Effluent (PE)		Waste Activated Sludge (WAS)			Activated Sludge Effluent (ASE)		Percent Removal†
		SS mg/L	Total Dye mg/L	SS mg/L	SOL Dye mg/L	RES Dye mg/g	SS mg/L	Total Dye mg/L	%
C.I. Acid Red 1									
Low Spike	Mean	190	0.90	1860	0.83	<0.01	25	0.88	2
	Std. Dev.	40	0.17	280	0.21	-	8	0.12	
	Number	8	8	8	8	8	8	8	
High Spike	Mean	260	4.43	1690	4.42	0.05	12	4.48	0
	Std. Dev.	58	0.75	66	0.98	0.02	6	0.91	
	Number	8	8	8	8	7	7	8	
C.I. Acid Red 88									
Low Spike	Mean	140	0.68	910	<0.02	0.10	14	0.04	94
	Std. Dev.	53	0.06	362	-	0.08	11	-	
	Number	6	5	6	6	6	6	6	
High Spike	Mean	150	3.96	1300	<0.01	0.08	22	<0.01	>99
	Std. Dev.	70	0.61	234	-	0.05	19	-	
	Number	6	6	6	6	6	6	6	
C.I. Acid Red 151									
Low Spike	Mean	140	0.56	910	0.08	2.89	14	0.17	70
	Std. Dev.	53	0.13	362	0.02	1.25	11	0.05	
	Number	6	5	6	6	6	6	6	
High Spike	Mean	150	3.64	1300	0.17	19.80	22	0.44	88
	Std. Dev.	70	0.76	234	0.03	5.30	19	0.10	
	Number	6	6	6	6	6	6	6	

TABLE 2. (Continued)

		Primary Effluent (PE)		Waste Activated Sludge (WAS)			Activated Sludge Effluent (ASE)		Percent Removal†
		SS mg/L	Total Dye mg/L	SS mg/L	SOL Dye mg/L	RES Dye mg/g	SS mg/L	Total Dye mg/L	%
C.I. Acid Red 337									
Low Spike	Mean	190	1.13	1490	0.68	1.15	7	0.93	18
	Std. Dev.	61	0.07	330	0.11	0.14	3	0.07	
	Number	5	5	5	5	5	5	4	
High Spike	Mean	220	5.06	1150	3.61	4.08	13	4.40	13
	Std. Dev.	105	0.39	252	0.48	0.87	2	0.51	
	Number	5	4	5	5	5	5	5	
C.I. Acid Yellow 151									
Low Spike	Mean	190	0.67	1860	0.26	0.46	25	0.48	28
	Std. Dev.	40	0.20	280	0.05	0.17	8	0.10	
	Number	8	8	8	8	8	8	8	
High Spike	Mean	260	4.05	1690	2.66	4.31	12	4.08	0
	Std. Dev.	58	0.60	66	0.58	1.23	6	0.44	
	Number	8	7	8	8	8	7	8	

*Units: SOL Dye = Concentration of soluble dye in filtrate
RES Dye = Mass of dye measured per mass of SS in the suspended residue

† Percent Removal = (PE - ASE)/PE X 100

Table 3. Mass Balance Summary

		Percent of Primary Effluent Dye Feed in:			
		Activated Sludge Effluent ASE %	Waste Activated Sludge (WAS) SOL %	RES %	ASE + WAS TOTAL %
C.I. Acid Blue 113					
	Low Spike	8	<1	68	76
	High Spike	17	1	49	67
C.I. Acid Orange 7					
Run 1	Low Spike	17	1	<1	19
	High Spike	4	<1	<1	4
Run 2	Low Spike	25	2	<1	27
	High Spike	12	1	<1	13
C.I. Acid Red 1					
	Low Spike	88	9	<1	97
	High Spike	92	9	<1	101
C.I. Acid Red 88					
	Low Spike	5	<1	1	7
	High Spike	<1	<1	<1	<1
C.I. Acid Red 151					
	Low Spike	28	1	44	73
	High Spike	11	<1	66	78
C.I. Acid Red 337					
	Low Spike	75	6	14	94
	High Spike	79	7	9	94
C.I. Acid Yellow 151					
	Low Spike	65	4	12	80
	High Spike	91	6	17	114

Table 4. Adsorption Isotherm Results

	Slope 1/n	Intercept* K	Correlation Coefficient r
C.I. Acid Blue 113	1.4	7.3	0.959
C.I. Acid Orange 7	1.2	0.11	0.975
C.I. Acid Red 1	24	1.0×10^{-17}	0.879
C.I. Acid Red 88	0.82	2.5	0.969
C.I. Acid Red 151	0.98	9.1	0.981
C.I. Acid Red 337	1.3	0.67	0.952
C.I. Acid Yellow 151	1.5	1.3	0.957

*When C_e = 1 mg/L

adsorption constants (1/n and K) and the linear regression correlation coefficient (r) and Figures 2 and 3 present these data graphically. The ASP data can be graphically presented on the isotherm plot as well. The value for C_e is directly related to the SOL values from the ASP data, each representing the equilibrium concentration of dye in solution (mg/L). Similarly, the value of X/M is directly related to the RES values from the ASP data, each representing the amount of dye adsorbed per unit weight of sludge solids (mg/g).

If the ASP data fall on or near the calculated line of best fit from the isotherm, then one could conclude that the isotherm provided a good estimate of the dye removal due to adsorption in the ASP studies. The adsorption isotherms provided reasonably good estimates of adsorption in the ASP studies for three of seven compounds. These were C.I. Acid Red 1, C.I. Acid Red 337 and C.I. Acid Yellow 151; the same three dyes for which no biodegradation occurred.

The ASP data for C.I. Acid Orange 7 and C.I. Acid Red 88 were not plotted on Figures 2 and 3, respectively, since many of the ASE values were reported as less than the detection limit (generally 0.01 mg/L) and thus, a finite ASE concentration could not be calculated. The ASP data from C.I. Acid Blue 113 and C.I. Acid Red 151 do not fall near the isotherm line. More apparent adsorption was calculated for each of these dyes in the ASP systems than was predicted by the isotherms. This significantly higher apparent adsorption cannot be explained at this time.

STATUS

No overall prediction about the fate of azo dyes in ASP systems can be made at this time as several additional compounds need to be tested before

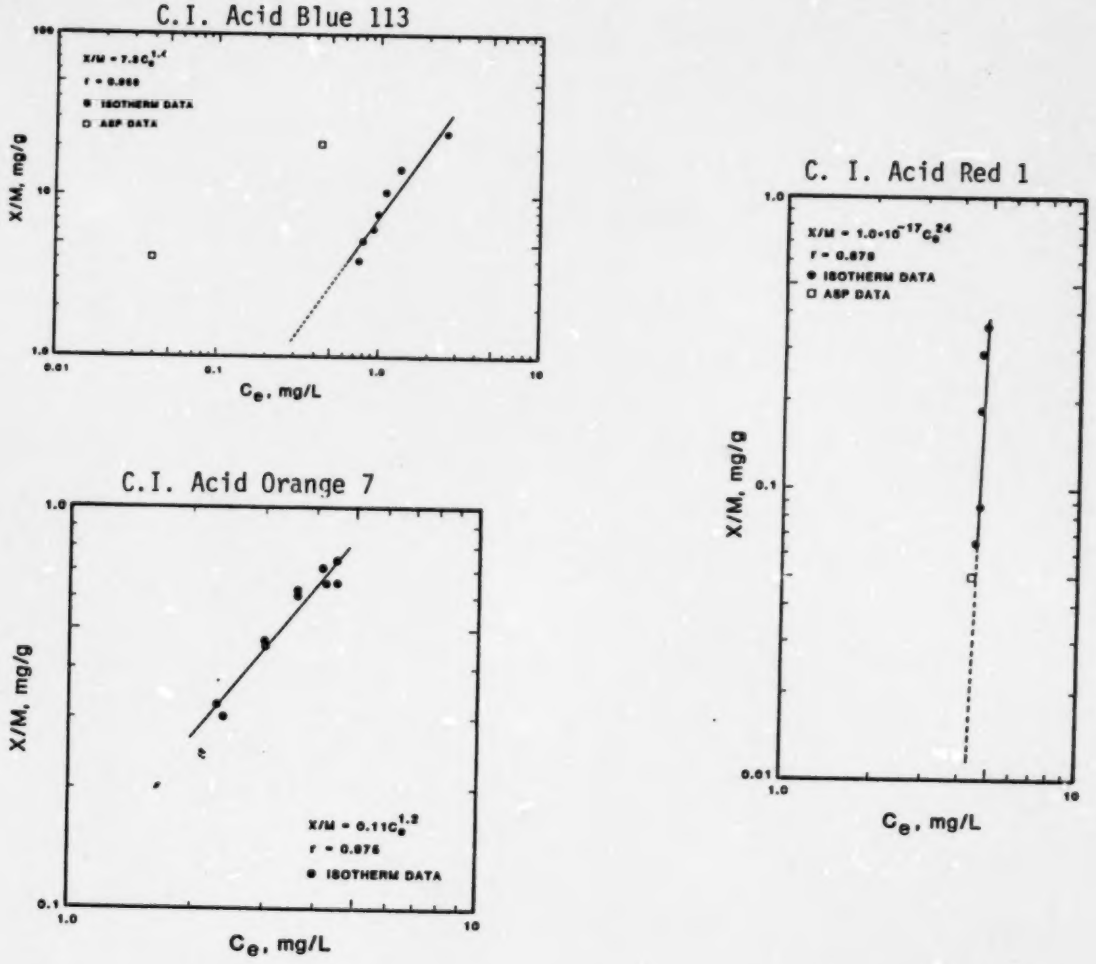


Figure 2. Adsorption Isotherms

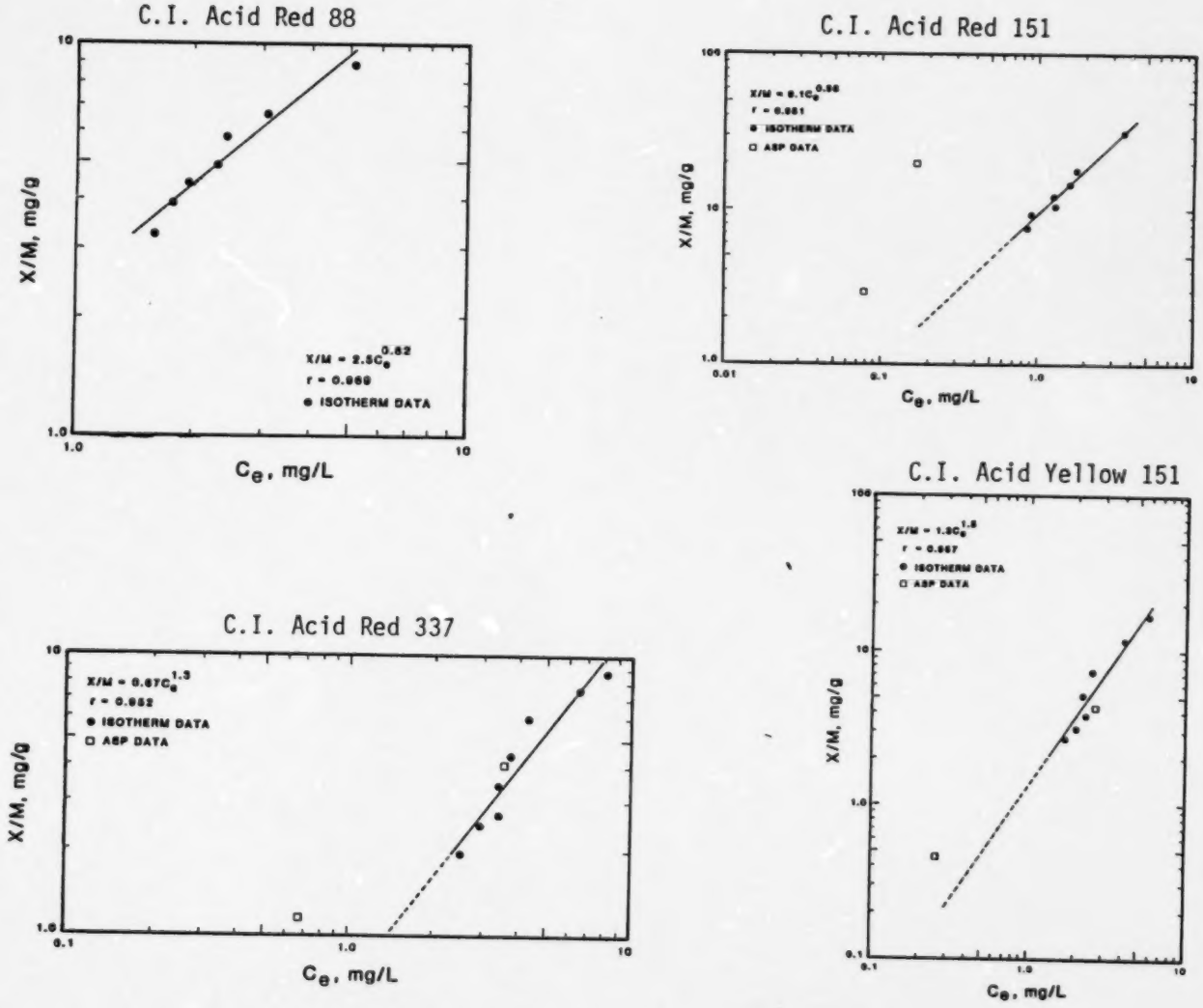


Figure 3. Adsorption Isotherms

generalities can be attempted. Once these additional data are available, relationships between physical and chemical properties, and the fate of the compounds will be investigated.

CONCLUSIONS

Based upon the data presented, the following conclusions can be made:

* C.I. Acid Red 1 appeared to be very nominally adsorbed but not biodegraded.
* C.I. Acid Red 337 and C.I. Acid Yellow 151 appear to be moderately adsorbed but not biodegraded.
* C.I. Acid Blue 113 and C.I. Acid Red 151 appear to be strongly adsorbed and possibly partially biodegraded.
* C.I. Acid Orange 7 and C.I. Acid Red 88 appear to be moderately adsorbed and significantly biodegraded.

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